Structural Effects of Cobalt-Amine Compounds on DNA Condensation

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ABSTRACT Light scattering and electron microscopy have been used to investigate the structural effects of the trivalent complexes hexaammine cobalt (III) chloride (Cohex), tris(ethylenediamine) cobalt(III) chloride (Coen), and cobalt(III) sepulchrate chloride (Cosep) on DNA condensation. These cobalt-amine compounds have similar ligand coordination geometries but differ slightly in size. Their hydrophobicity is in the order Cosep > Coen > Cohex, according to the numbers of methylene groups in these ligands. All of these compounds effectively precipitate DNA at high concentrations; but despite a lower surface charge density, Cosep condenses DNA twice as effectively as Coen or Cohex. UV and CD measurements of the supernatants of cobalt-amine/DNA solutions reveal a preferential binding of Δ-Coen over Λ-Coen to the precipitated DNA, but there is no chiral selectivity for Cosep. Competition experiments show that the binding strengths of these three cobalt-amine compounds to aggregated DNA are comparable. A charge neutralization of 88–90% is required for DNA condensation. Our data indicate that 1) electrostatic interaction is the main driving force for binding of multivalent cations to DNA; 2) DNA condensation is dependent on the structure of the condensing agent; and 3) the hydration pattern or polarization of water molecules on the surface of condensing agents plays an important role in DNA condensation and chiral recognition.

INTRODUCTION

Condensation or packaging of DNA is an important aspect of its function in transmitting and protecting genetic information. Because of the repeating units of negatively charged phosphate groups in its backbone, the packaging of DNA in viruses and chromosomes might be expected to be energetically unfavorable. Therefore, it is both biologically and physically interesting to understand the mechanism of DNA condensation (Bloomfield, 1997). In vitro, DNA condensation can be simply induced by adding condensing agents to aqueous DNA solutions. The simplest condensing agents are multivalent cations with valence ≥ 3. Schellman and co-workers were the first to demonstrate that the trivalent spermidine induces DNA condensation (Chattoraj et al., 1978; Gosule et al., 1978; Gosule and Schellman, 1976). Similar condensing behavior was found for other isovalent cations such as hexaammine cobalt and permethylated spermidine (Widom and Baldwin, 1980, 1983; Plum et al., 1990). Using two-ion counterion condensation theory (Manning, 1978), Wilson and Bloomfield (1979) defined ionic conditons and showed that 89–90% of the DNA charge must be neutralized for condensation to occur.

In addition to the nonspecific interaction of charge neutralization, specific interactions depending on the structure of the condensing agent have important impacts on DNA condensation (Bloomfield, 1996). The inorganic cation hexaammine cobalt condenses DNA fivefold more efficiently than spermidine (Widom and Baldwin, 1980), despite their equal valences. The morphology of condensates can also depend on condensing agent structure: a large fraction of rods rather than the more usual toroids was observed when spermidine was fully methylated (Plum et al., 1990).

To further explore the structural effects of condensing agents on DNA condensation, we here compare three cobalt-amine compounds: hexaammine cobalt (Cohex), tris-(ethylenediamine) cobalt (Coen), and cobalt sepulchrate (Cosep). The structural features and spectra of these three compounds are shown in Table 1 and Fig. 1.

All of these compounds are trivalent cations with a sexadentate coordination geometry. However, they differ in size, chemical composition, surface structures, and hydrophobicity. According to the numbers of methylene groups in these ligands, the size and hydrophobicity are in the order Cosep > Coen > Cohex. As trivalent cations, Coen and Cosep are expected to be capable of condensing DNA in aqueous solution as Cohex does, but their relative condensing powers are unknown. Because of their three ethylenediamine rings, Coen and Cosep are chiral molecules with Δ- and Λ-stereoisomers. We are particularly interested in their chiral selectivities and possible specific recognition of DNA in condensed phases.

MATERIALS AND METHODS

Chemicals and biochemicals

High-molecular-weight calf thymus DNA purchased from Pharmacia was dissolved in 1× TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and extracted several times with TE-saturated phenol. The aqueous layer was removed and precipitated with ethanol. DNA was resuspended and dialyzed against 4 M NaCl plus 5 mM sodium EDTA, twice against 0.4 M NaCl, and four times against Milli-Q water, and then lyophilized. For light scattering and EM experiments, DNA was prepared at 5 μg/ml in a solution containing 1 mM NaCl and 1 mM sodium cacodylate (pH 7.0). All solutions were filtered through 0.2-μm or 0.45-μm filters.

Cohex was purchased from Kodak, and racemic Coen and Cosep were from Aldrich. The extinction coefficients of Cohex, Coen, and Cosep at

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TABLE 1 Structures of the trivalent cobalt-amine complexes (chloride salts) used in this study

	Cohex	Coen	Cosep
Formula	$Co(NH_3)_6^{3+}$	$(H_2NCH_2CH_2NH_2)_3Co^{3+}$	$[(CH_2)_3N]_2(NHCH_2CH_2NH)_3Co^{3+}$
Composition	18 N-H, 0 C-H	12 N-H, 12 C-H	6 N-H, 24 C-H
Radius (Å)	3.0	3.1	3.3
Hydration	High	Intermediate	Low
Chirality	None	Racemic	Racemic

their maximum wavelengths (nm) are $\epsilon_{473} = 56.2\ M^{-1}\ cm^{-1}$, $\epsilon_{466} = 88\ M^{-1}\ cm^{-1}$, and $\epsilon_{472} = 109\ M^{-1}\ cm^{-1}$, respectively.

Light scattering

Total intensity light scattering was measured at a 90° scattering angle and room temperature (~25°C) after the introduction of condensing agents. A Lexel model 95 Ar laser was operated at a wavelength of 488 nm and a power of ~70 mW. The light scattering detected by the ITT FW-130 PMT was fed to a model BI-9000AT digital correlator (Brookhaven Instrument Corporation) for photon counting analysis.

Electron microscopy

Electron microscopy was performed 1 h after the addition of condensing agents. Carbon-coated 200 mesh grids (Electron Microscopy Sciences) were glow dischaged for 1.5 min at 6×10^{-5} Torr. One drop of sample solution was deposited on a grid and, after 1 min, stained with an aqueous solution of 1% uranyl acetate for 30 s. Excess liquid was withdrawn by holding filter paper to the edge of the grid. The sample was viewed on a Philips CM-12 TEM and recorded either on a photographic negative or via a CCD camera inserted into the TEM column. Images were then scanned into National Institutes of Health Image and digitized for the measurement of toroid dimensions.

UV-visible and CD spectroscopy

UV-visible spectroscopy and turbidimetry were carried out on a GBC spectrometer. CD spectra were collected on a Jasco 710 spectrometer. All experiments were performed at room temperature. Samples were held in 1-cm pathlength cells. Spectra of pure water solutions were taken as baselines and subtracted from the final supernatant spectra for measurements of relative binding of ligands to condensed DNA.

RESULTS

DNA condensation behavior

Comparisons of the onset of DNA condensation were obtained from total intensity light scattering measurements.

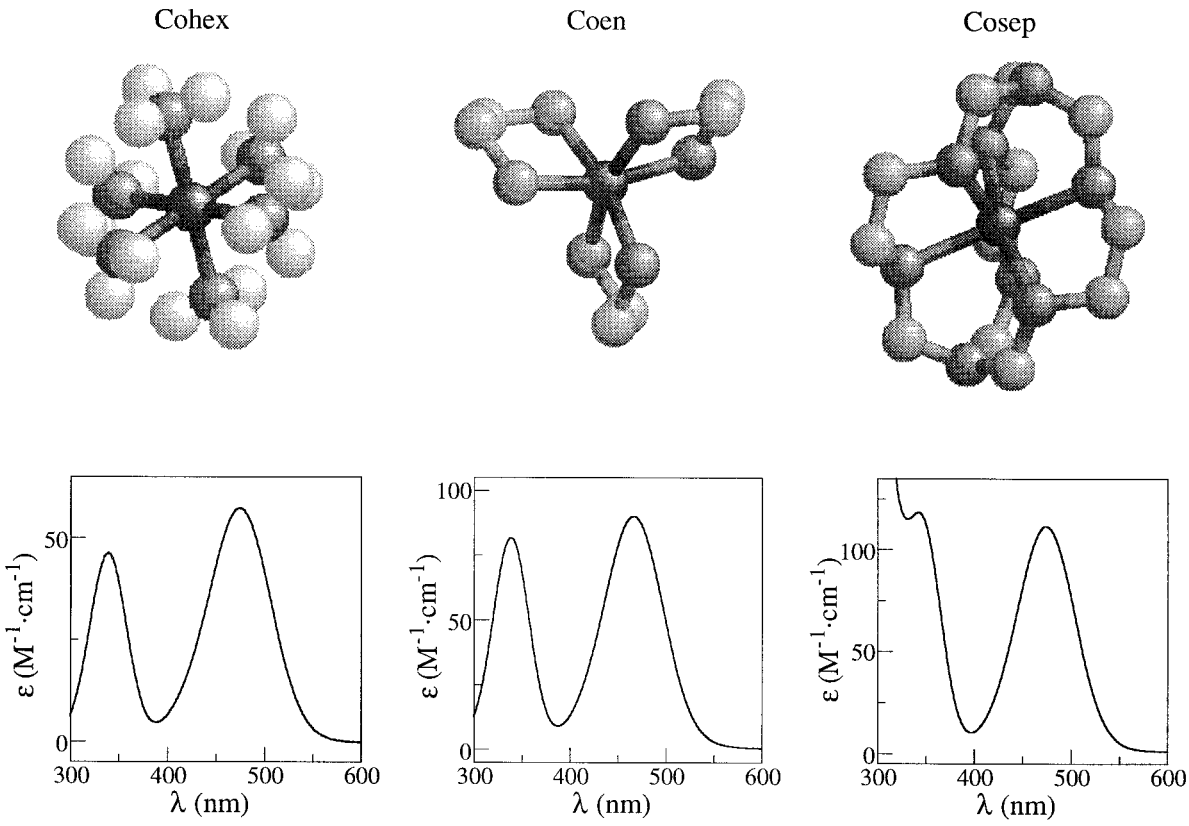

FIGURE 1 (*Top*) Structures of the cobalt-amine complexes used in this study. Hydrogen atoms are shown for Cohex, but not for Coen or Cosep. From the Cambridge Database, courtesy of Prof. Larry Que. (*Bottom*) UV-visible spectra of the compounds.

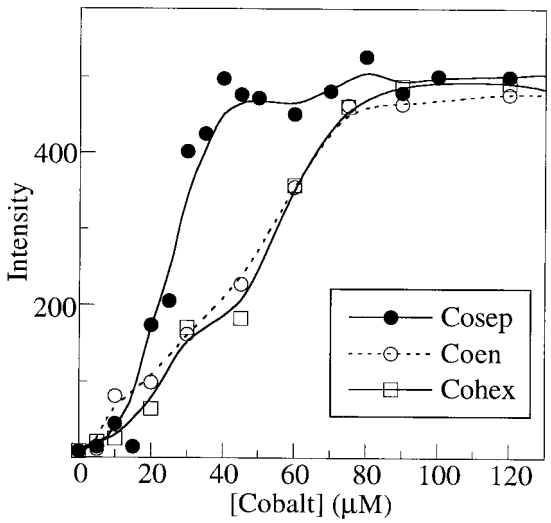

FIGURE 2 Light scattering intensity 2 h after the addition of Co-amine compounds. DNA samples (5 μg/ml) were prepared in solutions containing 1 mM sodium cacodylate and 1 mM NaCl (pH 7.0). The lines are not fits to the data, but are smoothed curves to guide the eye.

Fig. 2 shows light scattering intensities of DNA solutions plotted as a function of added cobalt-amine compound concentration. The scattering intensity in the presence of Cosep is higher than that in the presence of either Coen or Cohex. Critical concentrations are 25, 50, and 50 μM for Cosep, Coen, and Cohex, respectively. Cosep condenses DNA twice as effectively as Coen or Cohex, which have the same condensing power. Similar results (data not shown) were obtained by UV turbidity measurements at 388 nm for Coen and 397 nm for Cosep, wavelengths at which these compounds do not absorb light.

Fig. 3 shows that there are also some size and morphological differences between DNA condensed with Cosep relative to Cohex and Coen. With 50 μM Cohex or Coen, DNA condensates were predominantly toroids, with variable sizes but average inner and outer radii of 135 Å and 330 Å, respectively, whereas more unstructured aggregates than toroids were observed with 50 μM Cosep. At 25 μM Cosep, however, well-defined toroids and rods are abundant, with toroid sizes roughly double those in the presence of 50 μM Cosep.

Chiral selectivity of cobalt-amine compounds with aggregated DNA

All of these compounds effectively precipitate DNA at high concentrations. We mixed DNA with Coen or Cosep, with both ligand and DNA phosphate concentrations at 4.85 mM.

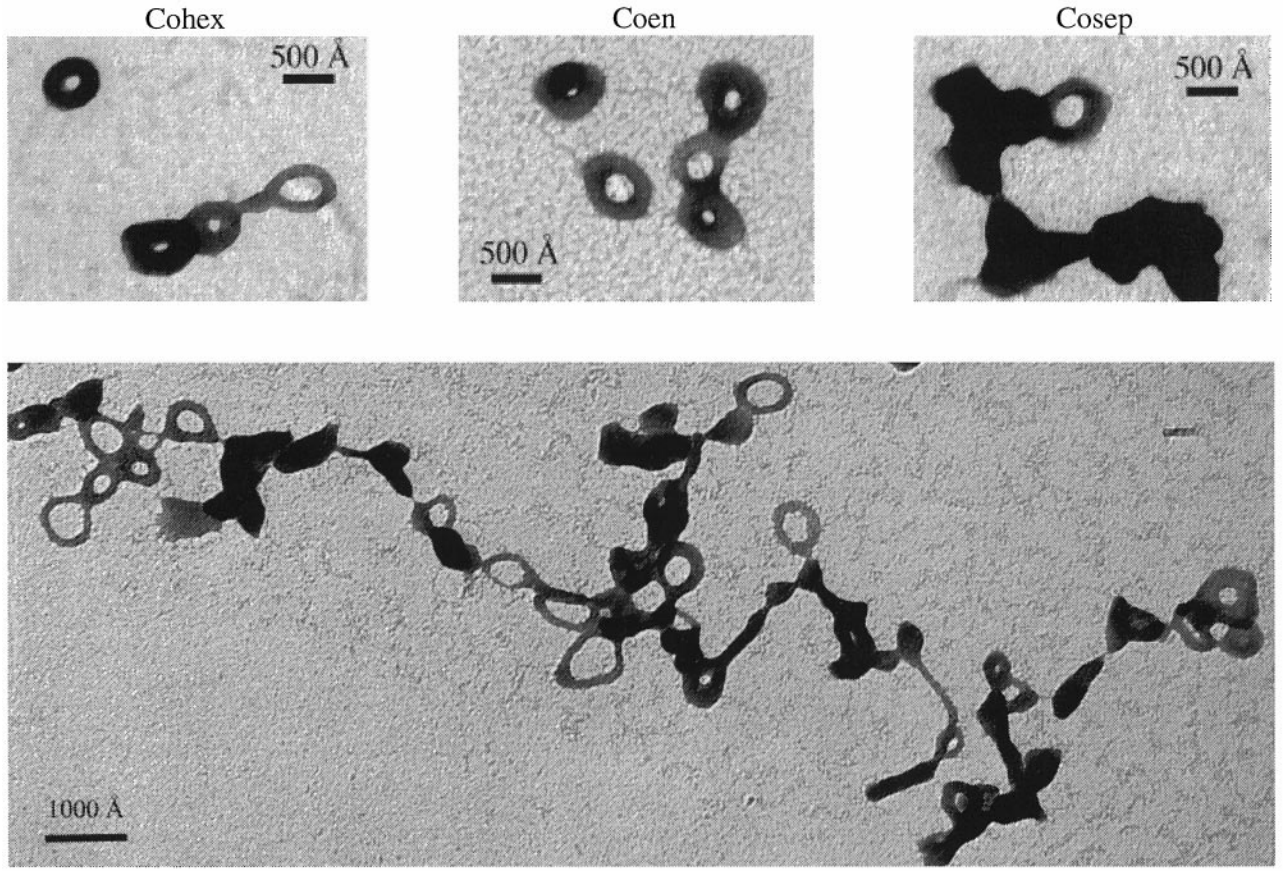

FIGURE 3 (*Top*) Condensed particles produced by calf thymus DNA 1 h after condensation with 50 μM Cohex, Coen, and Cosep. (*Bottom*) Condensed particles 1 h after condensation with 25 μM Cosep. [DNA] = 5 μg/ml in 1 mM sodium cacodylate, 1 mM NaCl (pH 7.0).

After high-speed sedimentation, DNA pelleted together with bound cobalt compounds at the bottom of Eppendorf tubes, whereas unbound ligands remained in the supernatants. Spectral measurements of the supernatants showed that ~3.42 mM of each cobalt compound remained in the supernatant, so 1.43 mM Coen or Cosep was bound to the condensed DNA. This corresponds to 88% charge neutralization of DNA phosphates, in excellent agreement with previous results (Wilson and Bloomfield, 1979).

CD measurements of the same samples are presented in Fig. 4. The Coen and Cosep used in our experiments were racemic mixtures, so no CD bands in the region of 400–600 nm are expected if the supernatants contain 50% Δ and 50% Λ stereoisomers. However, a positive band at 490 nm is observed from the Coen-DNA supernatant, which suggests that excess Λ-Coen remains in the supernatant. In other words, Δ-Coen preferentially binds to DNA aggregates. However, no such chiral selectivity was observed for Cosep.

Competitive binding of cobalt-amine compounds

To assess the relative strength of binding of these three cobalt compounds to DNA, we performed competitive binding experiments. A solution containing a mixture of two cobalt compounds, Coen/Cohex or Coen/Cosep, was used to precipitate DNA. The total cobalt concentration was kept at 2 mM, but the ratio of the ligands was varied. DNA phosphate concentrations were 3 mM. Fig. 5 shows the spectra of the supernatants from these experiments. The concentration of each cobalt compound in the supernatants was determined by deconvolution of the band at 400–550 nm into two bands, with a peak at 466.7 nm representing Coen, and a peak at 474.4 nm representing Cosep. The same analysis was applied to the spectra of Cohex/Coen supernatants, with a peak at 474.3 nm representing Cohex. Excellent curve fits were obtained for all deconvolutions.

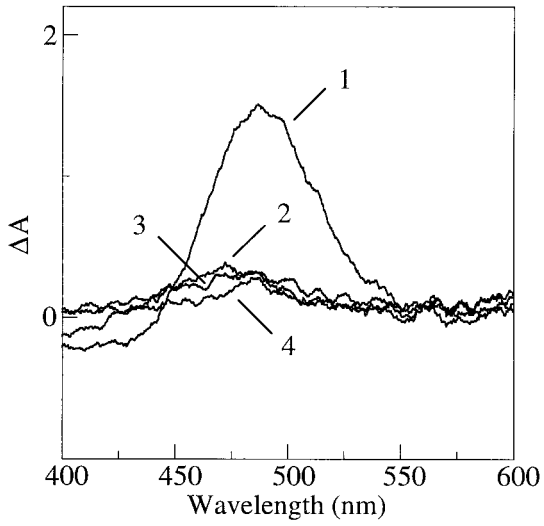

FIGURE 4 CD measurements on pure Coen and Cosep solutions and their supernatants. *1*: Coen/DNA supernatant; *2*: Cosep/DNA supernatant; *3*: Cosep; *4*: Coen. DNA phosphate and cobalt-amine concentrations were equal at 4.85 mM. CD spectra of supernatants were collected after 20 min of sedimentation at 14,000 rpm in Eppendorf tubes.

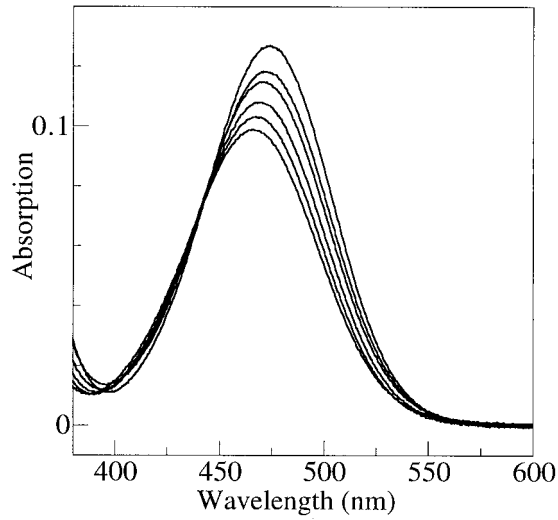

FIGURE 5 UV measurements of DNA supernatants in the presence of Coen and Cosep. All samples contained 3 mM DNA phosphate and a total of 2 mM cobalt compounds. The top and bottom curves are pure Cosep and pure Coen, respectively. The ratios Cosep:Coen in the mixed ligand solutions were 4/1, 3/2, 2/3, and 1/4 from top to bottom.

Fig. 6 shows the individual bound ligand concentrations and their sums as functions of initially added Coen concentration. The summed binding is independent of the initial ratio of the two compounds and corresponds to 90% charge neutralization of DNA phosphates. The values of the slopes from linear fits to the competitive binding curves are essentially equal, indicating that strengths of binding to aggregated DNA are comparable. This is true even though Cosep is a more effective condensing agent, a situation reminiscent of the observations that Cohex condenses DNA five times more effectively than spermidine (Widom and Baldwin, 1980), although the two compounds have equal binding constants for binding to DNA (Braunlin et al., 1982; Plum and Bloomfield, 1988).

DISCUSSION

Electrostatic interaction

Cosep, Coen, and Cohex are cobalt-amine complexes with a charge of +3. In this work we have shown that all of them can effectively condense DNA under conditions of low ionic strength in aqueous solution. Analysis of the trivalent cation concentration remaining in the supernatant indicates that 88–90% of the DNA phosphate charge has been neutralized upon DNA condensation, consistent with previous observations (Wilson and Bloomfield, 1979; Widom and Baldwin, 1980). The main driving force for DNA condensation is the strong electrostatic interaction between DNA and multivalent cations, leading to attractive correlated counterion fluctuations (Oosawa, 1968; Rouzina and

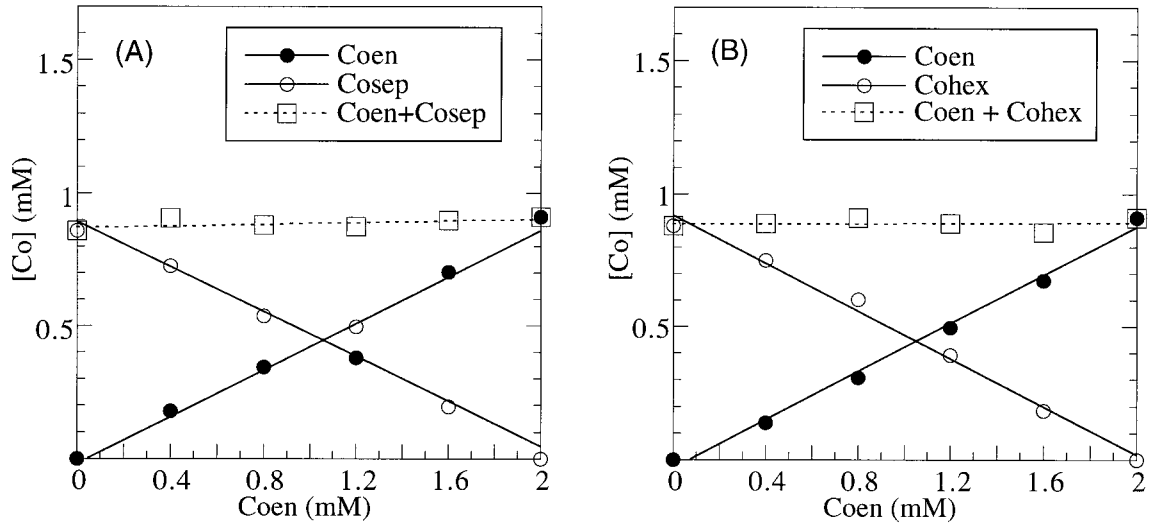

FIGURE 6 The individual bound ligand concentrations (○, ●) and their sum (□, ■) as functions of Coen concentration in mixed ligand solutions. DNAs were precipitated by a solution containing either Coen and Cosep (*A*) or Coen and Cohex (*B*). Total [Co] = 2 mM, [DNA-P] = 3 mM. The values of the slopes are ±4.3 in (*A*) and ±4.5 in (*B*).

Bloomfield, 1996; Gronbech-Jensen et al., 1997). Counterions also screen Coulombic repulsions between the DNA phosphates before condensation occurs. The similar sizes of toroidal condensates induced by these three compounds is a further reflection of the comparable balance between electrostatics and other energetic factors such as DNA bending and entropy loss (Bloomfield, 1991; Marquet and Houssier, 1991).

Nevertheless, we found that Cosep condenses DNA at a lower concentration than Cohex or Coen. Cosep is somewhat larger than Coen or Cohex, and electrostatics cannot explain why Cosep condenses DNA twice as effectively despite a lower surface charge density.

Hydration pattern

An alternative or supplementary explanation is hydration. DNA and cations are hydrated, even in the condensed state, and x-ray measurements of Cohex-precipitated DNA indicate a hexagonal packing with a ~7–8-Å separation between DNA surfaces (Leikin et al., 1991; Rau and Parsegian, 1992). The interactions between DNA and cations must be mediated by the intervening waters of hydration. Therefore, water content and, in particular, water polarization at DNA and cation surfaces must play an important role in DNA condensation (Leikin et al., 1993). Water content and polarization depend on molecular surface structures, so the surface character of a condensing ligand could be a key factor governing DNA condensation behavior.

Cosep has more hydrophobic CH_2 groups than Coen and Cohex. Six N-H groups are equally divided and localized in three small areas surrounded by hydrophobic CH_2 groups. Moreover, two unprotonated nitrogen atoms appear on Cosep's outpost surface. The hydration pattern on Cosep is expected to be significantly different from that of Coen or Cohex because of the addition of these two hydrophobic caps. For example, a hydration pattern such as lower water content, broken H-bond network, or flipped water polarization is possible on Cosep. Because the dipole moments of water molecules in clusters are extremely sensitive to multimeric water hydration structure (Gregory et al., 1997), any change in hydration at DNA-cation interfaces could affect DNA condensation. We speculate that the unique surface structure of Cosep structures its hydration pattern and thus enhances its condensing power.

Cation recognition of condensed DNA phases

DNA condensation assembles many molecules or molecular segments through side-by-side association. The surface spacing between DNA chains is usually only 7–8 Å. Higher order DNA mesophases, such as the cholesteric phase, are frequently found in aggregated DNA solutions (Livolant and Leforestier, 1996). In such a compact environment, a single cation will interact not just with a single DNA molecule, but with all surrounding DNAs through their hydration shells. As a consequence, chiral discrimination in DNA-chiral cation interactions could result simply from stereochemical complementarity. Recent NMR studies indicate that Δ-Coen preferentially binds to right-handed B-DNA, whereas Λ-Coen preferentially binds to left-handed DNA in solution (Xu et al., 1995). Under conditions that enhance their chiral identities by promoting the formation of cholesteric DNA mesophases, right-handed DNA molecules exhibit a higher affinity toward L-peptides (Reich et al., 1996).

Our data show that chiral discrimination in the interaction of condensed DNA phases with chiral cations can, but need not, occur. There was clear evidence of chiral selectivity with Coen, but Cosep did not show any chiral preference,

although it has the same core structure as Coen. A possible explanation is that the two tris(methylene) amino caps on Cosep alter the hydration pattern of recognition sites on its surface, thus diminishing chiral selectivity. Compared with Coen, Cosep is shaped more like a spherical ball than a propeller. Evidently, the hydrated surface structure of chiral ligands can modulate chiral selectivity. Unfortunately, because we do not have values for the molar ellipticities of the two stereoisomers, we cannot estimate the quantitative preference of Δ-Coen relative to Λ-Coen.

Cation motion

The unusual condensation behavior of Cosep suggests that binding of Cosep to DNA could be different from that of Coen and Cohex. Unprotonated nitrogens on Cosep surfaces might clash with negatively charged phosphates, forcing Cosep to align preferentially along the DNA helix axis once it is localized inside the assembling DNA lattice. This putative restriction on Cosep rotational motion could be another factor affecting its condensing power, because DNA condensation is essentially a competition between random thermal motion of hydrated molecules and DNA aggregation induced by multivalent cations.

CONCLUSIONS

In this paper, we have demonstrated that, although electrostatic interaction is the main driving force for binding of multivalent cations to DNA in solution, DNA condensation also depends on the structure of the condensing agent. Ligand recognition and consequent DNA condensation are affected by small changes in cation size, chemical composition, and surface structure. The hydration pattern or polarization of water molecules on the surface of condensing agents plays an important role in DNA condensation and chiral recognition.

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